Drug-Induced Pemphigus: Autoantibodies Directed Against the Pemphigus Antigen Complexes Are Present in Penicillamine and Captopril-Induced Pemphigus

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Pemphigus is an autoimmune blistering disease characterized by circulating autoantibodies directed against the keratinocyte cell surface. The two variants, pemphigus foliaceus and pemphigus vulgaris, can be distinguished at the molecular level by immunochemical studies. The large majority of patients with pemphigus develop the disease spontaneously; however, there is a small group of patients who develop pemphigus after treatment with certain medications, of which penicillamine and captopril are the best documented. Most patients with drug-induced pemphigus have circulating and/or tissue bound epidermal cell surface autoantibodies; however, the molecular specificity of these autoantibodies has not been studied.

We performed immunoprecipitation studies utilizing extracts of ^{125}I-labeled suction blister epidermis and the sera of three patients with drug-induced pemphigus foliaceus (two due to penicillamine and one due to captopril) and one patient with captopril-induced pemphigus vulgaris. We found that the three patients with drug-induced pemphigus foliaceus had circulating autoantibodies that are directed against the pemphigus foliaceus antigen complex and that the one patient with drug-induced pemphigus vulgaris had circulating autoantibodies that are directed against the pemphigus vulgaris antigen complex. This study demonstrates that autoantibodies from drug-induced pemphigus patients have the same antigenic specificity, on a molecular level, as do autoantibodies from other pemphigus patients. *J Invest Dermatol 96:273–276, 1991*

Pemphigus is an autoimmune blistering disease of the skin characterized by the presence of circulating autoantibodies directed against the keratinocyte cell surface [1]. Two clinical variants exist, pemphigus vulgaris and pemphigus foliaceus, and immunochemical studies have demonstrated that these variants can be distinguished at the molecular level. Patients with pemphigus vulgaris have circulating autoantibodies that bind to a characteristic complex of polypeptides of 210, 130, and 85 kD, whereas patients with pemphigus foliaceus have circulating autoantibodies that bind to another characteristic complex of polypeptides of 260, 160, and 85 kD [2]. The 160-kD polypeptide of the pemphigus foliaceus complex is desmoglein, a desmosomal core protein [3,4], and the common 85-kD polypeptide present in both complexes is plakoglobin, a desmosomal and adherens junction–associated molecule [5].

Although the large majority of patients with pemphigus do not have a history of any identifiable precipitating factor related to their disease, there is a small subgroup of patients who develop drug-induced pemphigus. Penicillamine, and the chemically related captopril, are the most frequently implicated agents [6–9], but several other medications have also been implicated [10–13].

Patients with drug-induced pemphigus fall into two categories; those whose disease remits after the drug is discontinued and those who, despite drug withdrawal, continue to have active disease, often requiring treatment with systemic glucocorticosteroids and/or immunosuppressive agents. It has been suggested that in patients with drug-induced pemphigus there is a correlation between those patients whose disease resolves soon after withdrawal of the offending drug and the lack of circulating or tissue-bound autoantibodies [14]. Recent studies demonstrating that penicillamine and captopril can both cause acantholysis in vitro, in the absence of autoantibody, lend support to this suggestion [14].

However, the majority of patients with drug-induced pemphigus have circulating and/or tissue bound antibodies [6] thus classifying them as autoimmune pemphigus. The purpose of this study was to determine if the antigenic specificities, at a molecular level, of autoantibodies from drug-induced pemphigus foliaceus and pemphigus vulgaris patients are the same as those of autoantibodies from patients with sporadically occurring pemphigus foliaceus and pemphigus vulgaris.

MATERIALS AND METHODS

Extracted epidermal proteins, derived from suction blister roofs of normal volunteers, were labeled with ^{125}I and subjected to immunoprecipitation according to a previously described protocol [2]. To summarize briefly, Nonidet P-40 extracted epidermal proteins were incubated with the different patient sera and antigen-antibody complexes were precipitated by protein A–bearing staphylocci. Sera from four drug-induced pemphigus patients (who are described in

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Table I. Clinical, Histologic, and Immunopathologic Findings in Patients with Drug-Induced Pemphigus

	Case 1	Case 2	Case 3	Case 4
Age/Sex	59 F	62 M	65 F	73 F
Drug	Penicillamine	Penicillamine	Captopril	Captopril
Clinical findings	Scaling patches on face, trunk, and extremities	Scaling patches on face, trunk, scalp, and extremities	Generalized polycyclic annular plaques with vesicles	Urticarial plaques, vesicles, and bullae on trunk, lower extremities, and axillae
Histology	Subcorneal acantholysis	Subcorneal and suprabasilar acantholysis	Subcorneal blister without acantholysis	Suprabasilar acantholysis
DIF[a]	Cell surface IgG, C3	Cell surface IgG	Cell surface IgG	Cell surface IgG, C3
IIF[a] Titer	> 80	> 80	10	40
Course	Cleared 3 months after stopping penicillamine	Improved after stopping penicillamine, but later required gold therapy	Improved after stopping captopril, but required systemic corticosteroid therapy	Cleared 2 months after stopping captopril
Reference	[15]	[15]		

[a] DIF direct immunofluorescence; IIF indirect immunofluorescence on monkey esophagus.

detail in *Results*) were tested. Sera from patients with clinically, histologically, and immunologically typical pemphigus foliaceus and pemphigus vulgaris were used as positive controls. These positive control sera demonstrated positive indirect immunofluorescence at titers greater than 80, with a cell-surface pattern on guinea pig esophagus or monkey esophagus. Normal human sera were used as negative controls. All extraction solutions, buffers, and washing solutions were supplemented with 2 mM $CaCl_2$, because previous studies revealed the presence of a calcium-sensitive epitope on the pemphigus foliaceus complex [3]. Immunoprecipitated proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and visualized by autoradiography with enhancing screens.

RESULTS

Patient Data The clinical, histologic, and immunopathologic features of the four cases are detailed in Table I. The two patients treated with penicillamine (patients 1 and 2) and one patient treated with captopril (patient 3) were diagnosed as most typical of pemphigus foliaceus, and the other patient treated with captopril (patient 4) was diagnosed as pemphigus vulgaris. Patients 1 and 2 have been previously reported [15]. Except for transient oral lesions in case 1, lasting only 5 d and unrelated to the course of her pemphigus, none of these patients had oral erosions. The histopathology was typical of pemphigus foliaceus in cases 1 and 3, and of pemphigus vulgaris in case 4. The histopathology of case 2 showed both subcorneal and suprabasilar acantholysis, but the patient had a clinical picture typical of pemphigus foliaceus and was thus classified. None of the biopsies showed eosinophilic spongiosis.

Penicillamine-Induced Pemphigus As expected, serum from a patient with typical, sporadically occurring, pemphigus foliaceus precipitated the pemphigus foliaceus complex, consisting of polypeptides of 260, 160, and 85 kD (Fig 1, *lane 1*). Sera from the two penicillamine-induced pemphigus foliaceus patients (patients 1 and 2) both precipitated the pemphigus foliaceus complex [Fig 1, *lanes 2* and *3* (patient 1, sera used obtained at different times) and Fig 1, *lane 4* (patient 2)], whereas normal human serum failed to precipitate any specific polypeptides (Fig 1, *lane 5*). Due to the presence of some atypical features for pemphigus foliaceus (patient 1 had transient oral erosions that are more commonly present in pemphigus vulgaris and patient 2 had one skin biopsy that revealed suprabasilar acantholysis as is normally seen in pemphigus vulgaris) we also performed immunoprecipitations utilizing extracts of ^{14}C-labeled cultured human keratinocytes to look for autoantibodies directed against the pemphigus vulgaris complex. This method, in which essentially all pemphigus vulgaris sera precipitate the 130- and 85-kD molecules [16], confirmed that the circulating antibodies from patients 1 and 2 did not recognize the pemphigus vulgaris antigen complex (data not shown).

These results demonstrate that these two patients with penicillamine-induced pemphigus have circulating antibodies that are directed against the pemphigus foliaceus complex.

Captopril-Induced Pemphigus As expected, serum from a patient with typical pemphigus foliaceus precipitated the pemphigus foliaceus complex consisting of polypeptides of 260, 160, and 85 kD (Fig 2, *lane 1*). Serum from patient 3 with captopril-induced pemphigus foliaceus also precipitated the pemphigus foliaceus

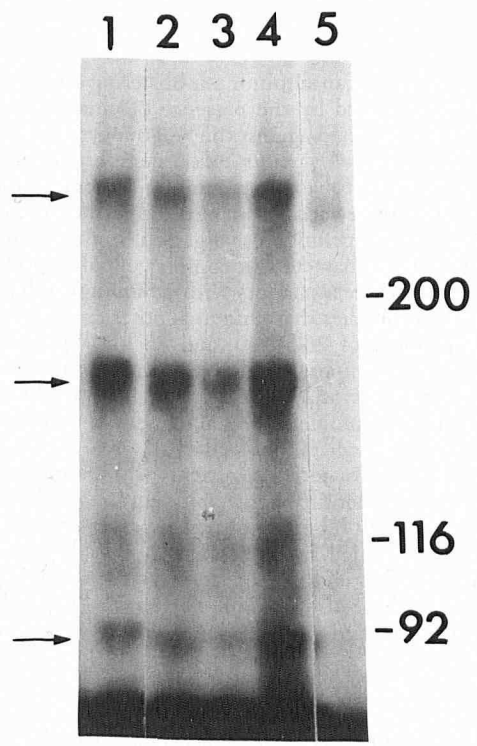

Figure 1. Patients with penicillamine-induced pemphigus foliaceus have circulating antibodies directed against the pemphigus foliaceus complex. *Lane 1,* serum from a patient with typical pemphigus foliaceus precipitated the pemphigus foliaceus complex (consisting of 260-, 160-, and 85-kD polypeptides as shown by arrows); *lanes 2* and *3,* serum from patient 1, obtained at different times, precipitated the pemphigus foliaceus complex; *lane 4,* serum from patient 2 precipitated the pemphigus foliaceus complex; *lane 5,* normal human serum control shows no specifically precipitated polypeptides. Molecular weight markers are as indicated in kD.

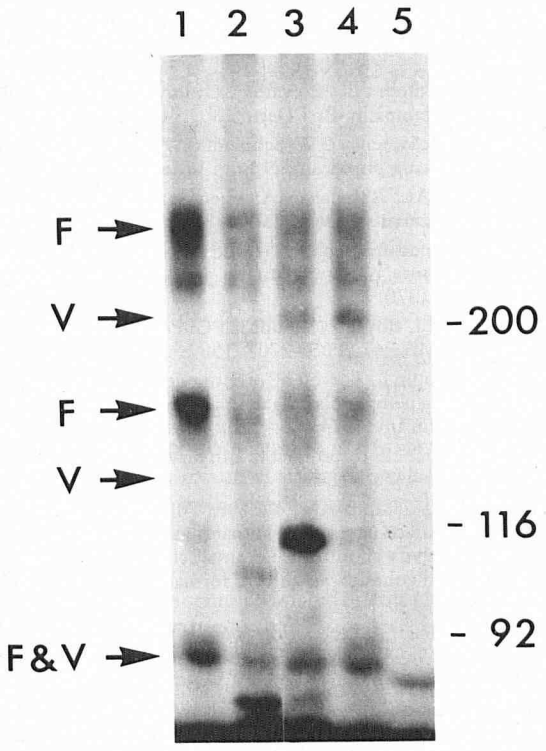

Figure 2. Patients with captopril-induced pemphigus have circulating antibodies directed against the pemphigus antigen complexes. *Lane 1,* serum of a patient with typical pemphigus foliaceus precipitated the pemphigus foliaceus complex (consisting of 260-, 160-, and 85-kD polypeptides as marked by Fs). *Lane 2,* serum of patient 3 with drug-induced pemphigus foliaceus precipitated the pemphigus foliaceus complex. *Lane 3,* serum of patient 4 with drug-induced pemphigus vulgaris precipitated the pemphigus vulgaris complex (marked by *V*s) and consisting of 210-kD, 130-kD (faint and difficult to visualize in this particular immunoprecipitation, as in the control pemphigus vulgaris immunoprecipitation in lane 4), and 85-kD polypeptides as well as the pemphigus foliaceus complex. This patient's serum also precipitated an intense approximately 110-kD band, the significance of which is not clear. *Lane 4,* serum of a patient with typical pemphigus vulgaris precipitated the pemphigus vulgaris complex as well as the pemphigus foliaceus complex. *Lane 5,* normal human serum control shows no specifically precipitated polypeptides. Molecular weight markers are as indicated in kD.

complex (Fig 2, *lane 2*). Serum from a patient with typical pemphigus vulgaris precipitated both the pemphigus vulgaris complex (marked by Vs), consisting of polypeptides of 210, 130, and 85 kD, as well as the pemphigus foliaceus complex (marked by the Fs) (Fig 2, *lane 4*). In the immunoprecipitation of this particular extract the 210-kD band is visualized much better than the 130-kD band. The 210- and 130-kD bands are characteristic of pemphigus vulgaris patients. The additional precipitation of the pemphigus foliaceus antigen complex is consonant with previous studies that have shown that, although pemphigus foliaceus sera never precipitate the pemphigus vulgaris antigen complex, approximately two thirds of pemphigus vulgaris sera precipitate both the pemphigus vulgaris and pemphigus foliaceus complexes when the immunoprecipitation is performed in the presence of 2 mM $CaCl_2$ [3]. Serum from patient 4 with captopril-induced pemphigus vulgaris precipitated both the pemphigus vulgaris complex as well as the pemphigus foliaceus complex (Fig 2, *lane 3*). The identity of the darkly staining band at approximately 110 kD in *lane 3* is not certain but it may represent the previously described minor 110-kD band of the pemphigus foliaceus complex [3]. However, its relevance or lack of relevance to drug-induced pemphigus vulgaris cannot be determined until more patients are tested by immunoprecipitation to determine if this 110-kD polypeptide is characteristic or simply an isolated finding in this one serum. Normal human serum failed to precipitate any of these pemphigus-specific polypeptides (Fig 2, *lane 5*). These results demonstrate that these two patients with captopril-induced pemphigus have circulating antibodies directed against the pemphigus antigen complexes.

Taken together these findings demonstrate that the antigenic specificities of drug-induced pemphigus foliaceus and vulgaris sera are the same as sera from sporadic cases of these diseases.

DISCUSSION

The major question addressed in this study is whether patients with drug-induced pemphigus develop the same autoimmune response as do patients with sporadic pemphigus. The results of this study, utilizing four patients with drug-induced pemphigus, demonstrate that both penicillamine and captopril can result in immunologically typical pemphigus vulgaris and pemphigus foliaceus.

Although the pathogenesis of drug-induced pemphigus is not well understood, several hypotheses have been proposed. Because penicillamine and captopril both have a similar chemical structure, with active sulfhydryl groups and similar toxicity profiles [17], it is reasonable to theorize that this active sulfhydryl group may play an important role in triggering drug-induced pemphigus. Recent studies have demonstrated that penicillamine and captopril can both cause acantholysis in epidermal organ culture in the absence of autoantibodies [14]. The binding of the drug could produce acantholysis by direct interaction of the sulfhydryl group with components of the epidermis (i.e., the pemphigus antigen complexes that contain sulfhydryl groups). This interaction could lead to a change in the conformation of these cell-surface antigens, rendering them immunogenic with resultant autoantibody formation. It is also possible that drug binding to the pemphigus antigen complexes could directly interfere with their normal function, which is thought to be epidermal cell adhesion [14]. However, this pathophysiologic mechanism would not require autoantibodies for blister formation. Finally, the observation that patients treated with penicillamine develop other autoimmune diseases, such as myasthenia gravis [17], suggests that these drugs may result in dysregulation of the immune response, with an increased tendency to develop autoantibodies. The heterogeneity of drug-induced pemphigus is underscored by the knowledge that different medications may induce this entity and that different clinical subtypes of pemphigus occur. It is therefore reasonable to conclude that different subtypes of drug-induced pemphigus may have different pathophysiologic mechanisms.

The circulating IgG antibody in pemphigus has been shown to be pathogenic and to induce acantholysis at the characteristic level in the epidermis in an organ culture model and when passively transferred to mice [18–20]. Although the mechanism by which these antibodies lead to acantholysis is not fully understood, there is evidence to suggest that proteinase activation may play an important part in sporadic pemphigus [18]. There is also a report that autoantibodies from one patient with penicillamine-induced pemphigus vulgaris may activate proteinases [21], suggesting that drug-induced pemphigus may share a common pathophysiologic pathway with sporadically occurring pemphigus. The present study, which demonstrates that patients with penicillamine and captopril-induced pemphigus have circulating autoantibodies directed against the pemphigus antigen complexes, supports the concept that drug-induced pemphigus may share with sporadic pemphigus a final common pathway of autoimmune damage to the epidermis.

Although all of the patients described here had both tissue bound and circulating pemphigus autoantibodies, two of the patients (1 and 4) had disease resolution soon after discontinuing the offending drug. This finding does not support the contention of Yokel et al that patients with drug-induced pemphigus whose disease resolves soon after drug withdrawal lack both tissue-bound and circulating autoantibodies [14].

The use of immunoprecipitation studies to evaluate the binding specificity, at the molecular level, of circulating autoantibodies has proved to be of great value in characterizing the autoimmune blis-

tering diseases of the skin. This report demonstrates the utility of this methodology in characterizing drug-induced pemphigus. Further studies of this type may lead to improved knowledge of other interesting variants of autoimmune blistering diseases.

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